EXHIBIT A

Improved disease resistance conferred by the RKS4 receptor

The ability of a subfamily of plant receptor-like kinases to confer disease resistance was tested in the model plant Arabidopsis thaliana. Arabidopsis Receptor Kinase like SERK (RKS) cDNA sequences were cloned into a CaMV 35s driven expression cassette and subsequently transformed in Arabidopsis. Transgenic plants were selected for the presence of a single transgene as well as for its overexpression. After sequential rounds of selection homozygous lines were subjected to disease resistance bioassays in order to assess changes in their response to several pathogens, as compared to wild-type control plants.

In addition to overexpression lines, T-DNA insertion lines were included in the same bioassays in order to study the effect of disrupted *RKS* gene function.

Several classes of pathogens were tested: the bacterium *Pseudomonas syringae*, the oomycete *Hyaloperonospora parasitica*, the biotrophic fungus *Fusarium oxysporum* and for some lines the necrotrophic fungus *Plectosphaerella cucumerina*. Resistance to the Western Flower Thrips *Frankliniella occidentalis* was also tested. Interesting results were obtained with several *RKS* genes, but more specifically with *RKS4* with which both overexpression and T-DNA insertion lines showed increased resistance (see Table 1 and Figure 2).

Detailed expression analysis of so-called RKS4-KO lines has shown that in the tested lines a truncated form of the *RKS4* gene was still expressed and strikingly to a rather high level (Figure 1). As a result of this change in gene activity disease resistance could also be obtained, similar to that observed with overexpression constructs. Overexpression constructs were made with truncated RKS4 coding sequences (RKS4Δ1, Δ2 and Δ3) and transformed in Arabidopsis in order to try mimicking the situation observed in the RKS4 'KO' lines. Changes in resistance were tested with the fungus Fusarium oxysporum and improved resistance could also be obtained by overexpression these truncated forms of RKS4, although less consistent results were observed (see Table 1 and Figure 2). This is most likely due to the fact that as opposed to the 'KO' lines the endogenous RKS4 is still normally expressed in these plants, which may reduce the effect of the introduced transgene.

Cytological studies were also performed along side the bioassay with the oomycete *H. parasitica* and revealed that resistance as observed in the RKS4 transgenics was associated with a physical reaction of the plant cells. Increased callose deposition could indeed be observed (Figure 3). This phenomenon is associated with a defense reaction of the plant as

also activated by the priming-inducing agent b-amino butyric acid (BABA), providing hereby additional proof of the defense reaction that is mediated by RKS4.

In addition RKS4 transgenic plants show no signs of fitness costs as often encountered when defense responses are constitutively activated through a transgenic approach. Plants even show increased organ size (Figure 4).

Line	Pseudomonas	Hyaloperonospora	Fusarium	Plectosphaerella
RKS5-OX1	-	nd	-	nd
RKS1-OX1	-	nd	-	nd
RKS1-OX2	-	nd	-	nd
RKS4-OX1	++	+	+	nd
RKS4-OX2	++	nd	++	nd
RKS4-OX3	nd	nd	++	nd
RKS4Δ1-OX1	nd	nd	+/-(?)	nd
RKS4Δ1-OX2	nd	nd	++	nd
RKS4Δ1-OX3	nd	nd	+/-(?)	nd
RKS4Δ2-OX1	nd	nd	+/-(?)	nd
RKS4Δ2-OX2	nd	nd	+/-(?)	nd
RKS4Δ3-OX1	nd	nd	+/-(?)	nd
RKS4Δ3-OX2	nd	nd	+/-(?)	nd
RKS4-KO1	++	+	-	+
RKS4-KO2	++	nd	-	+
RKS4-KO14	++	+	+	+
RKS4-KO4	nd	nd	+	+
RKS4-KO5	nd	nd	+	+
RKS4-KO6	nd	nd	-	+
RKS7-OX1	-	nd	-	nd
RKS7-KO1	nd	nd	+	+
RKS10-OX1	-	nd	+/-(?)	+
RKS10-OX2	nd	nd	+(?)	nd
RKS11-OX1	-	nd	-	nd
RKS11-KO1	nd	nd	+	+
RKS12-OX1	-	nd	+/-(?)	nd
RKS13-OX1	-	nd	-	nd
RKS14-OX1	nd	nd	nd	nd

Table 1. Overview of bioassays performed on Arabidopsis RKS transgenic plants. - no effect; + positive effect (to be repeated), (++: repeated experiment), nd: not done, (?) different result between experiments or unclear.

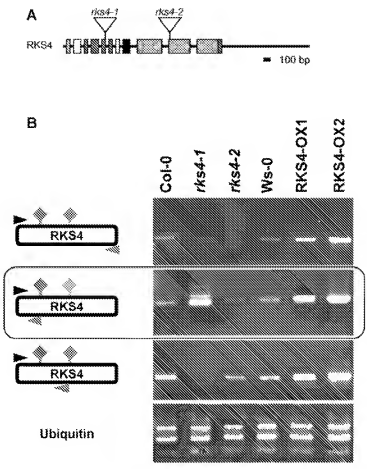

Figure 1. *RKS4* mRNA levels in knock-out and overexpression seedlings.

A. T-DNA insertion sites on the *RKS4* gene. **B.** RT-PCR analysis of the *RKS4* full-length messenger in 10 day-old seedlings from wild-type (Ws-0 and Col-0), two overexpression lines (RKS4-OX1 and RKS4-OX2) and two T-DNA insertion lines (*rks4-1* and *rks4-2*). A no template control (NTC) was included and equal amounts of cDNA template were assessed on the constitutive ubiquitin gene (Ubi). The position of the different oligonucleotides used within the RT-PCR reaction is indicated with respect to the different T-DNA integration sites on the right-hand side next to the corresponding Agarose Gel Electrophoresis pictures. Evidence for high expression of a truncated RKS4 messenger is shown in the second gel from the top (boxed).

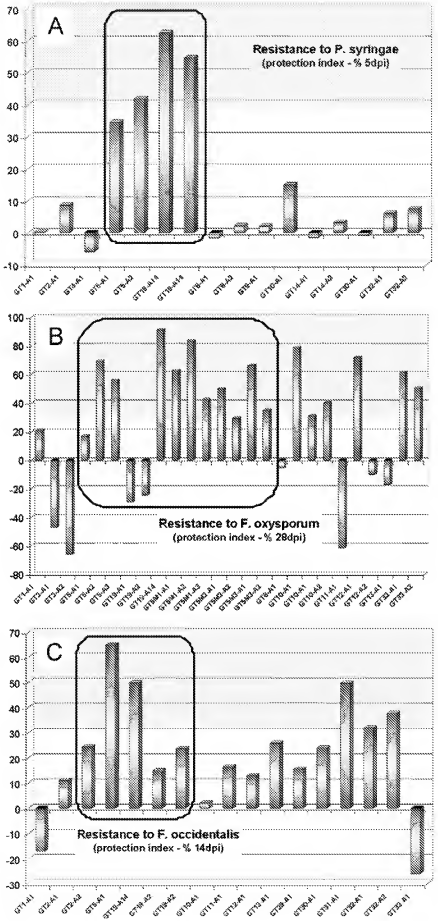
A
Resistance to P. syringae
(protection index - % 5dpi)
B
Resistance to F. oxysporum
(protection index - % 28dpi)
C
Resistance to F. occidentalis
(protection index - % 14dpi)

Figure 2. Sample overview of resistance assays performed on RKS transgenic plants.

Results shown in all panels represent the protection index obtained in each line which corresponds to the percentage of symptom-free leaves as compared to the wild-type. Lines overexpressing RKS4 (several variants) are boxed in the individual charts.

A. Resistance to *Pseudomonas syringae pv tomato DC3000 (Pst)*. Symptoms were scored on Arabidopsis rosette leaves 5 days post inoculation (dpi). Significant protection can be obtained in a number of cases, especially with the GT5 and GT19 lines that overexpress 2 different forms of the *RKS4* gene (full-length and truncated gene ('KO' lines), respectively).

B. Resistance to *Fusarium oxysporum f. sp. raphani*. Symptoms were scored on Arabidopsis rosette leaves 28 dpi. Significant protection can be obtained in an even larger number of cases than with Pst, and again especially with the GT5 and GT19 lines, but also with GT10 and GT12 in which the *RKS10* and RKS12 genes respectively are overexpressed. Overexpression lines with the truncated RKS4 constructs, RKS4Δ1-OX, Δ2-OX and Δ3-OX are shown as GT5M1, M2 and M3, respectively.

C. Resistance to *Frankliniella occidentalis* (Western Flower Thrips, also known as greenhouse thrips). Symptoms were scored on Arabidopsis rosette leaves 14 dpi. Again the highest levels of protection are mostly obtained with the GT5 and GT19 lines.

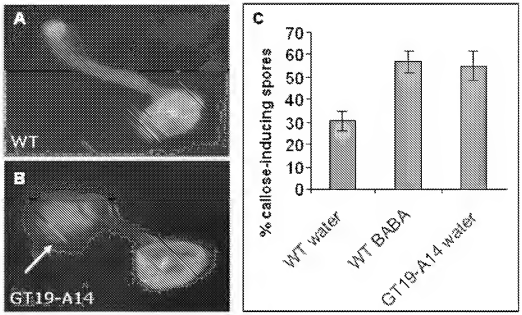

Figure 3. Enhanced callose deposition in RKS4 transgenic plants.

Callose deposition was observed on Arabidopsis leaves after infection with Hyaloperonospora *parasitica* essentially as described by Ton et al. (*Plant Cell* (2005) 17(3):987-999).

A. Example of germinating conidiospore in a wild-type leaf (WT). No callose deposition is observed as a result of the infection that can proceed normally.

B. Example of germinating conidiospore in a GT19-A14 (RKS4 'KO' line) leaf. Callose deposition (indicated by the arrow) is observed right in front of the elongating hyphae, which process is mechanically hindered by the callose plug.

C. Quantification of callose formation as a result of *H. parasitica* infection. Wild-type leaves were also treated with BABA as a control. The level of callose deposition in untreated GT19 plants is roughly the same as in BABA-treated plants, indicating that GT19 plants might as upon BABA treatment be better prepared to cope with *H. parasitica* infection.

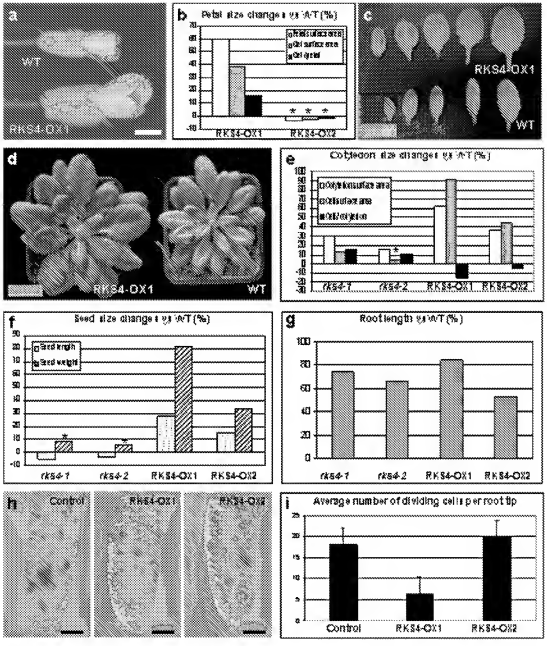

Figure 4. Morphological phenotypes induced by altered expression of RKS4. Histograms shown in panels (b), (e), (f) and (g) are based on measurements performed on plants with RKS4 altered expression and depict changes in percentages related to the corresponding wild-type (Col-0 for rks4-1 and -2; Ws-0 for RKS4-OX1 and 2). Statistical significance of the observed differences was analyzed by t-test and the * indicates that the measured differences are not statistically significant (i.e. p-value > 0.05).

(a) Increased flower size due to RKS4 overexpression (RKS4-OX1) versus wild-type Ws-0 (WT) (scale bar = 1 mm).

(b) Influence of RKS4 overexpression on petal and petal epidermis cell size. The number of cells/petal was obtained by dividing the mean of the petal surface area by the mean of the cell surface area.

(c) Altered leaf shape in rosettes of RKS4-OX1 plants (scale bar in cm).

(d) Overview of rosette shape and size in RKS4-OX1 and WT plants (scale bar in cm).

(e) Influence of RKS4 altered expression on cotyledon size based on measurements of the surface area of cotyledons and of their palisade mesophyll cells. The number of cells per cotyledon was obtained by dividing the mean surface area of the cotyledons by the one of the mesophyll cells.

(f) Influence of RKS4 altered expression on seed yield determined by seed length and weight measurement.

(g) Influence of RKS4 altered expression on root length as measured on 9 day-old seedlings grown on vertical plates.

(h-i) Changes in root tip mitotic activity caused by overexpression of RKS4. (h) From left to right: GUS positive/dividing cells in the root tip of a 7-d old seedling containing the pCDG construct (Colón-Carmona, A., You, R., Haimovitch-Gal, T. and Peter Doerner, O. (1999) Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J. 20, 503-508) alone; reduced number of dividing cells in the root tip of a 7-d old F1 seedling from a cross between RKS4-OX1 and pCDG; root tip of a 7-d old F1 seedling from a cross between RKS4-OX2 and pCDG (scale bar = 50 um). (i) Histogram of the average number of GUS positive cells per root tip in the main root (standard deviation indicated by the error bars).